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**Title:**           **TREATMENT OF FRUITS OR VEGETABLES  
WITH HYPERSENSITIVE RESPONSE ELICITOR  
TO CONTROL POSTHARVEST DISEASE OR  
DESICCATION**

**Inventors:**       **Zhong-Min Wei, Dewen Qiu, and Dean Remick**

**Docket No.:**     **21829/71 (EBC-005)**

# **TREATMENT OF FRUITS OR VEGETABLES WITH HYPERSENSITIVE RESPONSE ELICITOR TO INHIBIT POSTHARVEST DISEASE OR DESICCATION**

5                    This application claims benefit of U.S. Provisional Patent Application  
Serial No. 60/198,359, filed April 19, 2000, which is hereby incorporated by  
reference in its entirety.

## **FIELD OF THE INVENTION**

10                    The present invention relates to methods of treating fruits or vegetables  
to inhibit postharvest diseases and/or desiccation of harvested fruits or vegetables.

## **BACKGROUND OF THE INVENTION**

15                    Postharvest diseases are often extensions of disease occurring in the  
field or orchard. Brown rot of stone fruits (*Monilinia fructicola* (Wint.) Honey), for  
example, may cause blossom and twig blighting in the orchard. Infections in the  
orchard may not be visible at harvest if fruits are not refrigerated. *Colletotrichum*  
20 *gloeosporioides* (Penz.) Arx may attack blossoms or leaves and young fruit of citrus,  
avocados, mangos, papayas, and a wide range of other tropical and subtropical  
species; infections in developing fruit are usually latent, and rot lesions appear only at  
the onset of fruit ripening. *Pezicula malicorticis* (Jacks.) Nannfld. causes cankers of  
limbs of apples and pears; infections in developing fruit are latent, and active rotting  
25 usually commences only after the fruit has spent several months in storage and  
proceeds during -1°C storage because the organism is able to grow at very low  
temperatures. These fungi used as examples are able to penetrate the cuticle and  
epidermis of the fruit.

30                    Whether capable of being penetrated directly or not, wounds are often  
the usual means by which the fungus enters fruit. Cuts, punctures, bruises, and  
abrasions cannot be avoided completely during harvest and handling. If the cuticle  
and epidermis are broken, spores find nutrients and humidity in fresh wounds ideal for  
spore germination and colonization. Separation of fruits from the parent plant at  
harvest creates an unavoidable wound that encourages stem-end rots.

Rots developing at the blossom end usually involve prior colonization of floral parts. For example, *Botrytis* blossom-end rot (*B. cinerea*) sometimes occurs in Bartlett pears after a month or two in storage at -1°C. Initiation of rot in fruit flesh is associated with old styles and stamens retained within the fruit. Floral infections occur in the senescing floral parts at the end of blossoming. Mostly these floral parts are invaded by *Alternaria* spp. and common saprophytic fungi, but *B. cinerea* also is found occasionally. Not all fruits having *B. cinerea*-invaded floral parts rot in storage, but a significant percentage do. By contrast, test fruits remain free from *Botrytis* blossom-end rot if the old floral parts of developing fruits are free from *B. cinerea*. Rotting of fruits in storage is greatly reduced by a single orchard spray with a fungicide at the end of blossoming.

Contact infection, by which mycelia grow from a rotting fruit to contact and penetrate nearby fruit, is an especially serious aspect of some very common postharvest pathogens. The ever-enlarging "nest" of rotting fruit tied together by fungus mycelia will involve all fruit in a container, if given sufficient time.

Disease or threat of disease dictates in large measure the manner in which perishable fruits are handled. In recent decades, fruits have been shipped to increasingly greater distances from points of production. Exploitation of these distant markets, however, may offer large economic benefits only if the life of the commodity is stretched to its limit. Diseases and disorders ordinarily manageable during handling and transcontinental transit and marketing may be excessive when transoceanic marine transport of longer duration is involved. Similarly, the extension of marketing periods by storing fruits until they near the end of their physiological life may cause additional disease problems. Losses are especially serious if they occur in market areas, because the costs of sorting, packaging, cooling, storage, and transportation, which may greatly exceed production costs, have already been incurred. Of even greater long-term importance may be an impaired reputation leading to reduced future sales.

Postharvest diseases of fruit cause 15 to 25% losses yearly in the fruit industry worldwide and much of this is due to rot caused by microorganisms. Fungicides, which have been the primary means of controlling postharvest diseases, have come under scrutiny as posing potential oncogenic risks when applied to

processed foods. Thus, research efforts have been intensified to develop biological control procedures for postharvest diseases of fruits and vegetables that pose less risk to human health and the environment.

Considerable attention has been placed on assessing the use of antagonistic microorganisms as a viable alternative to the use of synthetic fungicides. Two basic approaches are available for using antagonistic microorganisms to control postharvest diseases. Naturally occurring antagonists that already exist on fruit and vegetable surfaces have been shown to control several rot pathogens on diverse commodities. Alternatively, artificially introduced antagonists have been shown to be effective in biologically controlling postharvest pathogens.

Since 1983, an explosion of research has occurred in the area of biological control of postharvest diseases by artificially introduced antagonists, mostly on fruit diseases (Janisiewicz, "Biological Control of Diseases of Fruit," In Biocontrol of Plant Diseases II, Mukergie et al. (ed.), CRC Press, Boca Raton, pp. 153-165 (1988) and Wilson et al., "Potential for Biological Control of Postharvest Plant Diseases," Plant Disease 69:375-378 (1985)). For example, rot on apples was controlled with yeast (Wisniewski et al., "Biological Control of Postharvest Diseases of Fruit: Inhibition of *Botrytis* Rot on Apples by an Antagonistic Yeast," Proc. Electron Microsc. Soc. Am. 46:290-91 (1988)), while brown rot in apricots was controlled with *Bacillus subtilis* (Pusey et al., "Postharvest Biological Control of Stone Fruit Brown Rot by *Bacillus subtilis*," Plant Dis. 68:753-56 (1984)). Mold incidence was reduced from 35% to 8% in lemon peel by a species of *Trichoderma* (De Matos, "Chemical and Microbiological Factors Influencing the Infection of Lemons by *Geotrichum candidum* and *Penicillium digitatum*," Ph.D. dissertation, University of California, Riverside, 106 pp. (1983)). Biocontrol of citrus rot pathogens was demonstrated with *Bacillus subtilis* (Singh et al., "*Bacillus subtilis* as a Control Agent Against Fungal Pathogens of Citrus Fruit," Trans. Br. Mycol. Soc. 83:487-90 (1984)). Such antagonists have various modes of action: antibiosis or competition for nutrients and space or both, induction of resistance in the host tissue, and direct interaction with the pathogen (Wilson et al., "Biological Control of Postharvest Diseases of Fruits and Vegetables: An Emerging Technology," Annu. Rev. Phytopathol. 27:425-441 (1989)).

While treatment with antagonistic bacterial or fungal species may be, at least to some extent, effective in controlling postharvest diseases, there are a number of factors which must be considered before this approach is used in commercial applications. First, the antagonists must be grown and maintained for use in treatments. This may result in significant expense and regulatory burdens depending on when and how frequently such antagonists would be applied. Also, it is questionable whether growers would want to maintain bioreactors for growing and propagating particular antagonist strains. Second, the efficacy of those antagonists may depend on storage conditions during shipment of harvested fruit. Some antagonists may not be able to tolerate variations in conditions during shipment, thereby allowing the pathogens to overcome any inhibitory effects of the antagonists. Given the above problems, it is not surprising that few of the antagonists reported to control plant pathogens have been successfully transferred from the laboratory into the field or postharvest environment.

Thus, there still exists a need to provide an effective, commercially viable method for treating fruits and vegetables to control postharvest diseases which avoids entirely or otherwise significantly reduces the need for fungicide treatments. In particular, it would be desirable to provide an effective, practicable treatment which presents little or no harm to humans or the environment.

The present invention is directed to overcoming these and other deficiencies in the art.

## SUMMARY OF THE INVENTION

The present invention relates to a method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit postharvest disease or desiccation.

A further aspect of the present invention relates to another method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out by providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic plant or transgenic plant produced from the transgenic plant

seed under conditions effective to inhibit a postharvest disease or desiccation in a fruit or vegetable harvested from the transgenic plant.

Another aspect of the present invention relates to a DNA construct that includes a DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a plant-expressible promoter operably coupled 5' to the DNA molecule, the promoter being effective to transcribe the DNA molecule in fruit or vegetable tissue, and a 3' regulatory region operably coupled to the DNA molecule, wherein expression of the DNA molecule in fruit or vegetable tissue imparts to a fruit or vegetable resistance against postharvest disease or desiccation. Also disclosed are expression systems, host cells, and transgenic plants which contain a heterologous DNA construct of the present invention.

By the present invention, the hypersensitive response elicitor protein or polypeptide can be used to inhibit or otherwise control postharvest diseases (i.e., caused by pathogens) in fruits or vegetables. Likewise, such treatment can also inhibit postharvest desiccation of treated fruits or vegetables. In achieving these objectives, the present invention enables produce growers, warehouse packers, shippers, and suppliers to process, handle, and store fruits and vegetables with reduced losses caused by postharvest disease and desiccation. As a result, the cost of bringing fruits and vegetables from the field to the consumer can be reduced. Importantly, the quality of the treated fruits or vegetables is improved.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit postharvest disease or desiccation.

A further aspect of the present invention relates to another method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out by providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic plant or transgenic plant produced from the transgenic plant

seed under conditions effective to inhibit a postharvest disease or desiccation in a fruit or vegetable harvested from the transgenic plant.

For use in accordance with these methods, suitable hypersensitive response elicitor proteins or polypeptides are those derived from a wide variety of bacterial and fungal pathogens, preferably bacterial pathogens.

Exemplary hypersensitive response elicitor proteins and polypeptides from bacterial sources include, without limitation, the hypersensitive response elicitors derived from *Erwinia* species (e.g., *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, etc.), *Pseudomonas* species (e.g., *Pseudomonas syringae*, *Pseudomonas solanacearum*, etc.), and *Xanthomonas* species (e.g., *Xanthomonas campestris*). In addition to hypersensitive response elicitors from these Gram-negative bacteria, it is possible to use elicitors derived from Gram-positive bacteria. One example is the hypersensitive response elicitor derived from *Clavibacter michiganensis* subsp. *sepedonicus*.

Exemplary hypersensitive response elicitor proteins or polypeptides from fungal sources include, without limitation, the hypersensitive response elicitors (i.e., elicitors) from various *Phytophthora* species (e.g., *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, *Phytophthora citrophthora*, etc.).

Preferably, the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia chrysanthemi*, *Erwinia amylovora*, *Pseudomonas syringae*, or *Pseudomonas solanacearum*.

A hypersensitive response elicitor protein or polypeptide from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

Met	Gln	Ile	Thr	Ile	Lys	Ala	His	Ile	Gly	Gly	Asp	Leu	Gly	Val	Ser
1				5					10					15	
Gly	Leu	Gly	Ala	Gln	Gly	Leu	Lys	Gly	Leu	Asn	Ser	Ala	Ala	Ser	Ser
			20					25					30		
Leu	Gly	Ser	Ser	Val	Asp	Lys	Leu	Ser	Ser	Thr	Ile	Asp	Lys	Leu	Thr
		35					40					45			
Ser	Ala	Leu	Thr	Ser	Met	Met	Phe	Gly	Gly	Ala	Leu	Ala	Gln	Gly	Leu
	50					55					60				
Gly	Ala	Ser	Ser	Lys	Gly	Leu	Gly	Met	Ser	Asn	Gln	Leu	Gly	Gln	Ser
65					70					75				80	

5 Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys  
85 90 95  
Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp  
100 105 110  
10 Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln  
115 120 125  
Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met  
130 135 140  
15 Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly  
145 150 155 160  
Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly  
165 170 175  
Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu  
180 185 190  
20 Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala  
195 200 205  
Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val  
210 215 220  
25 Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp  
225 230 235 240  
Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp  
245 250 255  
Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys  
260 265 270  
30 Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln  
275 280 285  
Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr  
290 295 300  
Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala  
305 310 315 320  
Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala  
325 330 335  
Asn Ala

35 This hypersensitive response elicitor protein or polypeptide has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine. This *Erwinia chrysanthemi* hypersensitive response elicitor protein or polypeptide is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 2 as follows:

40 cgattttacc cgggtgaacg tgctatgacc gacagcatca cggtattcga caccgttacg 60  
gcgtttatgg ccgcgatgaa ccggcatcag gcggcgcgct ggtcgccgca atccggcgctc 120  
gatctggtat ttcagtttg ggcacccggg cgtgaactca tgatgcagat tcagccgggg 180  
cagcaatatc ccggcatggt gcgcacgctg ctcgctcgtc gttatcagca ggccggcagag 240



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 15  
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 25  
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tgcgatggct gccatctgtg cctgaacggc agcgatgtat tgatcctctg gtggccgctg 300  
 ccgtcggatc ccggcagtta tccgcagggtg atcgaacggt tgtttgaact ggcggggaatg 360  
 acgttgccgt cgctatccat agcaccgacg gcgcgtccgc agacagggaa cggacgcgcc 420  
 cgatcattaa gataaaggcg gcttttttta ttgcaaaacg gtaacgggtga ggaaccgttt 480  
 caccgtcggc gtcactcagt aacaagtatc catcatgatg cctacatcgg gatcggcgctg 540  
 ggcacccgtt gcagatactt ttgcgaacac ctgacatgaa tgaggaaacg aaattatgca 600  
 aattacgata aaagcgca tccggcggtga tttgggcgctc tccggtctgg ggctgggtgc 660  
 tcagggactg aaaggactga attccgcggc ttcacgcgtg ggttccagcg tggataaact 720  
 gagcagcacc atcgataagt tgacctccgc gctgacttcg atgatgtttg gcggcgcgct 780  
 ggcgcgaggg ctgggcgcca gctcgaagg gctggggatg agcaatcaac tgggccagtc 840  
 tttcggcaat ggcgcgagg gtgcgagcaa cctgctatcc gtaccgaaat ccggcgcgga 900  
 tgcgttgtca aaaatgtttg ataaagcgct ggacgatctg ctgggtcatg acaccgtgac 960  
 caagctgact aaccagagca accaaactggc taattcaatg ctgaacgcca gccagatgac 1020  
 ccagggtaat atgaatgcgt tccgcagcgg tgtgaacaac gcactgtcgt ccattctcgg 1080  
 caacggtctc ggccagtcga tgagtggctt ctctcagcct tctctggggg caggcggtt 1140  
 gcagggcctg agcggcgcggt gtgcattcaa ccagttgggt aatgccatcg gcatggcgct 1200  
 ggggcagaat gctgcgtga gtgcgttgag taacgtcagc acccacgtag acggtaacaa 1260  
 ccgccacttt gtagataaag aagatcgcg catggcgaaa gagatcggcc agtttatgga 1320  
 tcagtatccg gaaatattcg gtaaaccgga ataccagaaa gatggctgga gttcgccgaa 1380  
 gacggacgac aaatcctggg ctaaagcgct gagtaaaccg gatgatgacg gtatgaccgg 1440  
 cgccagcatg gacaaattcc gtcaggcgat gggatatgatc aaaagcgcg tggcggtgga 1500  
 taccggcaat accaacctga acctgcgtgg cgcggggcgg gcatcgctgg gtatcgatgc 1560  
 ggctgtcgtc ggcgataaaa tagccaacat gtcgctgggt aagctggcca acgcctgata 1620  
 atctgtgctg gcctgataaa gcggaaacga aaaaagagac ggggaagcct gtctcttttc 1680  
 ttattatgcg gtttatgagg ttacctggac cggttaatca tcgtcatcga tctggtacaa 1740  
 acgcacattt tcccgttcat tcgcgtcgtt acgcgccaca atcgcgatgg catcttcctc 1800  
 gtcgctcaga ttgcgcggct gatggggaac gccgggtgga atatagagaa actcgccggc 1860  
 cagatggaga cagctctgcg ataaatctgt gccgtaacgt gtttctatcc gcccttttag 1920  
 cagatagatt gcggtttcgt aatcaacatg gtaatgcggg tccgcctgtg cgccggccgg 1980  
 gatcaccaca atattcatag aaagctgtct tgcacctacc gtatcgcggg agataccgac 2040  
 aaaatagggc agtttttgcg tggatatccgt ggggtgttcc ggccctgaaa tcttgagttg 2100  
 gttcgtcatc atctttctcc atctgggcga cctgatcggt t 2141

35 The above nucleotide and amino acid sequences are disclosed and further described in  
 U.S. Patent No. 5,850,015 to Bauer et al. and U.S. Patent No. 5,776,889 to Wei et al.,  
 which are hereby incorporated by reference in their entirety.

A hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 3 as follows:

5	Met	Ser	Leu	Asn	Thr	Ser	Gly	Leu	Gly	Ala	Ser	Thr	Met	Gln	Ile	Ser
	1				5					10					15	
	Ile	Gly	Gly	Ala	Gly	Gly	Asn	Asn	Gly	Leu	Leu	Gly	Thr	Ser	Arg	Gln
				20					25					30		
10	Asn	Ala	Gly	Leu	Gly	Gly	Asn	Ser	Ala	Leu	Gly	Leu	Gly	Gly	Gly	Asn
			35					40					45			
	Gln	Asn	Asp	Thr	Val	Asn	Gln	Leu	Ala	Gly	Leu	Leu	Thr	Gly	Met	Met
		50					55					60				
	Met	Met	Met	Ser	Met	Met	Gly	Gly	Gly	Gly	Leu	Met	Gly	Gly	Gly	Leu
	65					70					75					80
15	Gly	Gly	Gly	Leu	Gly	Asn	Gly	Leu	Gly	Gly	Ser	Gly	Gly	Leu	Gly	Glu
					85					90					95	
	Gly	Leu	Ser	Asn	Ala	Leu	Asn	Asp	Met	Leu	Gly	Gly	Ser	Leu	Asn	Thr
				100					105					110		
20	Leu	Gly	Ser	Lys	Gly	Gly	Asn	Asn	Thr	Thr	Ser	Thr	Thr	Asn	Ser	Pro
			115					120						125		
	Leu	Asp	Gln	Ala	Leu	Gly	Ile	Asn	Ser	Thr	Ser	Gln	Asn	Asp	Asp	Ser
		130					135					140				
	Thr	Ser	Gly	Thr	Asp	Ser	Thr	Ser	Asp	Ser	Ser	Asp	Pro	Met	Gln	Gln
	145					150					155					160
25	Leu	Leu	Lys	Met	Phe	Ser	Glu	Ile	Met	Gln	Ser	Leu	Phe	Gly	Asp	Gly
					165					170					175	
	Gln	Asp	Gly	Thr	Gln	Gly	Ser	Ser	Ser	Gly	Gly	Lys	Gln	Pro	Thr	Glu
				180					185					190		
30	Gly	Glu	Gln	Asn	Ala	Tyr	Lys	Lys	Gly	Val	Thr	Asp	Ala	Leu	Ser	Gly
			195					200					205			
	Leu	Met	Gly	Asn	Gly	Leu	Ser	Gln	Leu	Leu	Gly	Asn	Gly	Gly	Leu	Gly
		210					215					220				
	Gly	Gly	Gln	Gly	Gly	Asn	Ala	Gly	Thr	Gly	Leu	Asp	Gly	Ser	Ser	Leu
	225					230					235					240
35	Gly	Gly	Lys	Gly	Leu	Gln	Asn	Leu	Ser	Gly	Pro	Val	Asp	Tyr	Gln	Gln
					245					250					255	
	Leu	Gly	Asn	Ala	Val	Gly	Thr	Gly	Ile	Gly	Met	Lys	Ala	Gly	Ile	Gln
				260					265					270		
40	Ala	Leu	Asn	Asp	Ile	Gly	Thr	His	Arg	His	Ser	Ser	Thr	Arg	Ser	Phe
			275					280						285		
	Val	Asn	Lys	Gly	Asp	Arg	Ala	Met	Ala	Lys	Glu	Ile	Gly	Gln	Phe	Met
		290					295					300				
	Asp	Gln	Tyr	Pro	Glu	Val	Phe	Gly	Lys	Pro	Gln	Tyr	Gln	Lys	Gly	Pro
	305					310					315					320
45	Gly	Gln	Glu	Val	Lys	Thr	Asp	Asp	Lys	Ser	Trp	Ala	Lys	Ala	Leu	Ser
					325					330					335	

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Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn  
340 345 350  
Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn  
355 360 365  
5 Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp  
370 375 380  
Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu  
385 390 395 400  
Gly Ala Ala

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This hypersensitive response elicitor protein or polypeptide has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor protein or polypeptide has substantially no cysteine. The hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* is more fully described in Wei, Z-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference in its entirety. The DNA molecule encoding this hypersensitive response elicitor protein or polypeptide has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

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35

aagcttcggc atggcacgtt tgaccgttgg gtcggcaggg tacgtttgaa ttattcataa 60  
gaggaatacg ttatgagtct gaatacaagt gggctgggag cgtcaacgat gcaaatttct 120  
atcggcgggtg cgggcggaaa taacgggttg ctgggtacca gtcgccagaa tgctgggttg 180  
ggtggcaatt ctgcactggg gctgggcggc ggtaatcaaa atgataccgt caatcagctg 240  
gctggcttac tcaccggcat gatgatgatg atgagcatga tgggcgggtg tgggctgatg 300  
ggcgggtggct taggcgggtg cttaggaat ggcttgggtg gctcaggtg cctgggcgaa 360  
ggactgtcga acgcgctgaa cgatatgtta ggcggttcgc tgaacacgct gggctcgaaa 420  
ggcggcaaca ataccacttc aacaacaaat tccccgctg accaggcgct ggggtattaac 480  
tcaacgtccc aaaacgacga ttccacctcc ggcacagatt ccacctcaga ctccagcgac 540  
ccgatgcagc agctgctgaa gatgttcagc gagataatgc aaagcctgtt tggatgatggg 600  
caagatggca cccagggcag ttctctctggg ggcaagcagc cgaccgaagg cgagcagaac 660  
gcctataaaa aaggagtcac tgatgcgctg tcgggcctga tgggtaatgg tctgagccag 720  
ctccttggca acgggggact gggaggtggt cagggcggta atgctggcac gggctctgac 780  
ggttcgtcgc tgggcggcaa agggctgcaa aacctgagcg ggccgggtgga ctaccagcag 840  
ttaggtaacg ccgtgggtac cggtatcggg atgaaagcgg gcattcaggc gctgaatgat 900  
atcggtagcg acaggcacag ttcaaccctg tctttcgtca ataaaggcga tcgggcgatg 960  
gcgaaggaaa tcggtcagtt catggaccag tatcctgagg tggttggcaa gccgcagtac 1020  
cagaaaggcc cgggtcagga ggtgaaaacc gatgacaaat catgggcaaa agcactgagc 1080  
aagccagatg acgacggaat gacaccagcc agtatggagc agttcaacaa agccaagggc 1140

atgatcaaaa ggcccatggc gggtgatacc ggcaacggca acctgcaggc acgcggtgcc 1200  
ggtggttctt cgctgggtat tgatgccatg atggccggtg atgccattaa caatatggca 1260  
cttggaagc tgggcgcggc ttaagctt 1288

5 The above nucleotide and amino acid sequences are disclosed are further described in  
U.S. Patent No. 5,849,868 to Beer et al. and U.S. Patent No. 5,776,889 to Wei et al.,  
which are hereby incorporated by reference in their entirety.

Another hypersensitive response elicitor protein or polypeptide derived  
from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No.  
10 5 as follows:

Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu  
1 5 10 15  
Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser  
15 20 25 30  
Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala  
35 40 45  
Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly  
50 55 60  
Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly  
20 65 70 75 80  
Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro  
85 90 95  
Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu  
25 100 105 110  
Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gly Gln Ile Gly Asp  
115 120 125  
Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp  
130 135 140  
30 Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala  
145 150 155 160  
Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser  
165 170 175  
35 Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro  
180 185 190  
Val Ser Thr Phe Ser Pro Pro Ser Thr Pro Thr Ser Pro Thr Ser Pro  
195 200 205  
Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro  
210 215 220  
40 Val Thr Asp His Pro Asp Pro Val Gly Ser Ala Gly Ile Gly Ala Gly  
225 230 235 240  
Asn Ser Val Ala Phe Thr Ser Ala Gly Ala Asn Gln Thr Val Leu His  
245 250 255

Asp Thr Ile Thr Val Lys Ala Gly Gln Val Phe Asp Gly Lys Gly Gln  
260 265 270  
Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn  
275 280 285  
5 Gln Lys Pro Leu Phe Ile Leu Glu Asp Gly Ala Ser Leu Lys Asn Val  
290 295 300  
Thr Met Gly Asp Asp Gly Ala Asp Gly Ile His Leu Tyr Gly Asp Ala  
305 310 315 320  
10 Lys Ile Asp Asn Leu His Val Thr Asn Val Gly Glu Asp Ala Ile Thr  
325 330 335  
Val Lys Pro Asn Ser Ala Gly Lys Lys Ser His Val Glu Ile Thr Asn  
340 345 350  
Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp  
355 360 365  
15 Thr Asn Leu Ser Val Asp Asn Val Lys Ala Lys Asp Phe Gly Thr Phe  
370 375 380  
Val Arg Thr Asn Gly Gly Gln Gln Gly Asn Trp Asp Leu Asn Leu Ser  
385 390 395 400  
20 His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser  
405 410 415  
Glu Gly Leu Asn Val Asn Thr Ser Asp Ile Ser Leu Gly Asp Val Glu  
420 425 430  
Asn His Tyr Lys Val Pro Met Ser Ala Asn Leu Lys Val Ala Glu  
435 440 445

This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It is also heat stable, protease sensitive, and suppressed by inhibitors of plant metabolism. The protein or polypeptide of the present invention has a predicted molecular size of ca. 4.5 kDa. The DNA molecule encoding this hypersensitive response elicitor protein or polypeptide has a nucleotide sequence corresponding to SEQ. ID. No. 6 as follows:

atgtcaattc ttacgcttaa caacaatacc tegtctctgc cgggtctgtt ccagtcaggg 60  
ggggacaacg ggcttggtgg tcataatgca aattctgcgt tggggcaaca acccatcgat 120  
35 cggcaaacca ttgagcaa at ggctcaatta ttggcggaac tggttaaagtc actgctatcg 180  
ccacaatcag gtaatgcggc aaccggagcc ggtggcaatg accagactac aggagttggt 240  
aacgctggcg gcctgaacgg acgaaaaggc acagcaggaa ccactccgca gtctgacagt 300  
cagaacatgc tgagtgagat gggcaacaac gggctggatc aggccatcac gcccgatggc 360  
cagggcgggc ggcagatcgg cgataatcct ttactgaaag ccatgctgaa gcttattgca 420  
40 cgcgatgatg acggccaaag cgatcagttt ggccaacctg gtacgggcaa caacagtgcc 480  
tcttcgggta cttcttcac tggcggttcc ccttttaacg atctatcagg ggggaaggcc 540  
ccttcgggca actccccctt cggcaactac tctcccgta gtaccttctc acccccatcc 600  
acgccaacgt cccctacctc accgcttgat ttcccttctt ctcccaccaa agcagccggg 660

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ggcagcacgc cggtaaccga tcatacctgac cctgttggtgta ggcggggcat cggggccgga 720  
aattcgggtgg ccttcaccag cgccggcgct aatcagacgg tgctgcatga caccattacc 780  
gtgaaagcgg gtcaggtggt tgatggcaaa ggacaaacct tcaccgccgg ttcagaatta 840  
ggcgatggcg gccagtctga aaaccagaaa ccgctgttta tactggaaga cggtgccagc 900  
ctgaaaaacg tcaccatggg cgacgacggg gcggatggta ttcatacttta cggtgatgcc 960  
aaaatagaca atctgcacgt caccaacgtg ggtgaggacg cgattaccgt taagccaaac 1020  
agcgcggggca aaaaatccca cggtgaaatc actaacagtt ccttcgagca cgcctctgac 1080  
aagatcctgc agctgaatgc cgatactaac ctgagcgttg acaacgtgaa ggccaaagac 1140  
tttggtactt ttgtacgcac taacggcggg caacagggta actgggatct gaatctgagc 1200  
catatcagcg cagaagacgg taagtctctg ttcgttaaaa gcgatagcga ggggctaaac 1260  
gtcaatacca gtgatattct actgggtgat gttgaaaacc actacaaagt gccgatgtcc 1320  
gccaacctga aggtggctga atga 1344

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent Application Serial No. 09/120,927 to Beer et al., which is hereby incorporated by reference in its entirety.

A hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 7 as follows:

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Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met  
1 5 10 15  
Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser  
20 25 30  
Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met  
35 40 45  
Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala  
50 55 60  
Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val  
65 70 75 80  
Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe  
85 90 95  
Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met  
100 105 110  
Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu  
115 120 125  
Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met  
130 135 140  
Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro  
145 150 155 160  
Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe  
165 170 175

5 Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile  
     180                    185                    190  
 Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly  
     195                    200                    205  
 10 Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser  
     210                    215                    220  
 Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser  
     225                    230                    235                    240  
 Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp  
     245                    250                    255  
 Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val  
     260                    265                    270  
 Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln  
     275                    280                    285  
 15 Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala  
     290                    295                    300  
 Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala  
     305                    310                    315                    320  
 20 Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg  
     325                    330                    335  
 Asn Gln Ala Ala Ala  
     340

25 This hypersensitive response elicitor protein or polypeptide has a molecular weight of  
 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine.  
 Further information about the hypersensitive response elicitor derived from  
*Pseudomonas syringae* is found in He, S. Y., et al., "*Pseudomonas syringae* pv.  
*syringae* Harpin<sub>PS</sub>: a Protein that is Secreted via the Hrp Pathway and Elicits the  
 Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), which is hereby  
 30 incorporated by reference in its entirety. The DNA molecule encoding this  
 hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide  
 sequence corresponding to SEQ. ID. No. 8 as follows:

35 atgcagagtc tcagtccttaa cagcagctcg ctgcaaacc cggcaatggc ccttgtcctg 60  
 gtacgtcctg aagccgagac gactggcagt acgtcgagca aggcgcttca ggaagttgtc 120  
 gtgaagctgg ccgaggaact gatgcgcaat ggtcaactcg acgacagctc gccattggga 180  
 aaactgttgg ccaagtcgat ggccgcagat ggcaaggcgg gcggcggtat tgaggatgtc 240  
 atcgtgctgc tggacaagct gatccatgaa aagctcggtg acaacttcgg cgcgtctgctg 300  
 gacagcgctt cgggtaccgg acagcaggac ctgatgactc aggtgctcaa tggcctggcc 360  
 40 aagtcgatgc tcgatgatct tctgaccaag caggatggcg ggacaagctt ctccgaagac 420  
 gatatgccga tgctgaacaa gatcgcgag ttcattggatg acaatccccg acagtttccc 480

aagccggact cgggctcctg ggtgaacgaa ctcaaggaag acaacttcct tgatggcgac 540  
 gaaacggctg cgttccgttc ggcaactcgac atcattggcc agcaactggg taatcagcag 600  
 agtgacgctg gcagtctggc agggacgggt ggaggtcttg gcactccgag cagtttttcc 660  
 aacaactcgt ccgtgatggg tgatccgctg atcgacgcca ataccgggtcc cggtgacagc 720  
 5 ggcaataccc gtggtgaagc ggggcaactg atcggcgagc ttatcgaccg tggcctgcaa 780  
 tcggtattgg ccggtggtgg actgggcaca cccgtaaaca ccccgagac cggtacgtcg 840  
 gcgaatggcg gacagtcgc tcaggatcct gatcagttgc tgggcggctt gctgctcaag 900  
 ggcttgagg caacgctcaa ggatgccggg caaacaggca ccgacgtgca gtcgagcgct 960  
 gcgcaaatcg ccaccttgct ggtcagtacg ctgctgcaag gcacccgcaa tcaggctgca 1020  
 10 gcctga 1026

The above nucleotide and amino acid sequences are disclosed and further described in  
 U.S. Patent No. 5,708,139 to Collmer et al. and U.S. Patent No. 5,776,889 to Wei et  
 al., which are hereby incorporated by reference in their entirety.

15 Another hypersensitive response elicitor protein or polypeptide derived  
 from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID.  
 No. 9 as follows:

20 Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu  
 1 5 10 15  
 Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly  
 20 25 30  
 Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly  
 35 40 45  
 25 Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val  
 50 55 60  
 Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile  
 65 70 75 80  
 30 Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr  
 85 90 95  
 Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln  
 100 105 110  
 Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser  
 115 120 125  
 35 Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Asp Thr  
 130 135 140  
 Pro Ser Ala Thr Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly  
 145 150 155 160  
 40 Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly  
 165 170 175  
 Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr  
 180 185 190

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	Pro	Gln	Ile	Thr	Pro	Gln	Leu	Ala	Asn	Pro	Asn	Arg	Thr	Ser	Gly	Thr	
			195					200					205				
	Gly	Ser	Val	Ser	Asp	Thr	Ala	Gly	Ser	Thr	Glu	Gln	Ala	Gly	Lys	Ile	
		210					215					220					
5	Asn	Val	Val	Lys	Asp	Thr	Ile	Lys	Val	Gly	Ala	Gly	Glu	Val	Phe	Asp	
	225					230					235					240	
	Gly	His	Gly	Ala	Thr	Phe	Thr	Ala	Asp	Lys	Ser	Met	Gly	Asn	Gly	Asp	
					245					250					255		
10	Gln	Gly	Glu	Asn	Gln	Lys	Pro	Met	Phe	Glu	Leu	Ala	Glu	Gly	Ala	Thr	
				260					265					270			
	Leu	Lys	Asn	Val	Asn	Leu	Gly	Glu	Asn	Glu	Val	Asp	Gly	Ile	His	Val	
		275						280					285				
	Lys	Ala	Lys	Asn	Ala	Gln	Glu	Val	Thr	Ile	Asp	Asn	Val	His	Ala	Gln	
		290					295					300					
15	Asn	Val	Gly	Glu	Asp	Leu	Ile	Thr	Val	Lys	Gly	Glu	Gly	Gly	Ala	Ala	
	305					310					315					320	
	Val	Thr	Asn	Leu	Asn	Ile	Lys	Asn	Ser	Ser	Ala	Lys	Gly	Ala	Asp	Asp	
					325				330						335		
20	Lys	Val	Val	Gln	Leu	Asn	Ala	Asn	Thr	His	Leu	Lys	Ile	Asp	Asn	Phe	
				340					345					350			
	Lys	Ala	Asp	Asp	Phe	Gly	Thr	Met	Val	Arg	Thr	Asn	Gly	Gly	Lys	Gln	
			355					360					365				
	Phe	Asp	Asp	Met	Ser	Ile	Glu	Leu	Asn	Gly	Ile	Glu	Ala	Asn	His	Gly	
		370					375					380					
25	Lys	Phe	Ala	Leu	Val	Lys	Ser	Asp	Ser	Asp	Asp	Leu	Lys	Leu	Ala	Thr	
	385					390				395						400	
	Gly	Asn	Ile	Ala	Met	Thr	Asp	Val	Lys	His	Ala	Tyr	Asp	Lys	Thr	Gln	
				405					410						415		
30	Ala	Ser	Thr	Gln	His	Thr	Glu	Leu									
				420													

This protein or polypeptide is acidic, glycine-rich, lacks cysteine, and is deficient in aromatic amino acids. The DNA molecule encoding this hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ.

ID. No. 10 as follows:

	tccacttcgc	tgattttgaa	attggcagat	tcatagaaac	gttcaggtgt	ggaaatcagg	60
	ctgagtgcgc	agatttcggt	gataagggtg	tggtactggt	cattgttggt	catttcaagg	120
	cctctgagt	cggtgcggag	caataccagt	cttcctgctg	gcgtgtgcac	actgagtcgc	180
40	aggcataggc	atttcagttc	cttgcggttg	ttgggcatat	aaaaaaagga	acttttaaaa	240
	acagtgcgat	gagatgccgg	caaaacggga	accggtcgct	gcgctttgcc	actcacttcg	300
	agcaagctca	accccaaaca	tccacatccc	tatcgaacgg	acagcgatac	ggccacttgc	360
	tctggtaaac	cctggagctg	gcgtcggtcc	aattgcccac	ttagcgaggt	aacgcagcat	420
	gagcatcggc	atcacacccc	ggccgcaaca	gaccaccacg	ccactcgatt	tttcggcgct	480

aagcggcaag agtcctcaac caaacacggt cggcgagcag aacactcagc aagcgatcga 540  
 cccgagtgca ctgttggttcg gcagcgacac acagaaagac gtcaacttcg gcacgcccga 600  
 cagcaccgtc cagaatccgc aggacgccag caagcccaac gacagccagt ccaacatcgc 660  
 taaattgata agtgcattga tcatgtcgtt gctgcagatg ctcaccaact ccaataaaaa 720  
 5 gcaggacacc aatcaggaac agcctgatag ccaggctcct ttccagaaca acggcgggct 780  
 cggtacaccg tcggccgata gcggggggcg cggtacaccg gatgcgacag gtggcggcgg 840  
 cggtgatacg ccaagcgcaa caggcggtgg cggcggtgat actccgaccg caacaggcgg 900  
 tggcggcagc ggtggcggcg gcacacccac tgcaacaggt ggccggcagcg gtggcacacc 960  
 cactgcaaca ggcggtggcg aggggtggcgt aacaccgcaa atcactccgc agttggccaa 1020  
 10 ccctaaccgt acctcaggta ctggctcggt gtcggacacc gcaggttcta ccgagcaagc 1080  
 cggcaagatc aatgtggtga aagacaccat caaggctcggc gctggcgaag tctttgacgg 1140  
 ccacggcgca accttcactg ccgacaaaatc tatgggtaac ggagaccagg gcgaaaatca 1200  
 gaagcccatg ttcgagctgg ctgaaggcgc tacgttgaag aatgtgaacc tgggtgagaa 1260  
 cgaggtcgat ggcattccag tgaaagccaa aaacgctcag gaagtcacca ttgacaacgt 1320  
 15 gcatgcccag aacgtcgggtg aagacctgat tacgggtcaaa ggcgagggag gcgcagcggg 1380  
 cactaatctg aacatcaaga acagcagtgc caaagggtgca gacgacaagg ttgtccagct 1440  
 caacgccaac actcacttga aaatcgacaa cttcaaggcc gacgatttcg gcacgatggt 1500  
 tcgcaccaac ggtggcaagc agtttgatga catgagcatc gagctgaacg gcatcgaagc 1560  
 taaccacggc aagttcgccc tgggtgaaaag cgacagtgc gatctgaagc tggcaacggg 1620  
 20 caacatcgcc atgaccgacg tcaaacacgc ctacgataaa acccaggcat cgacccaaca 1680  
 caccgagctt tgaatccaga caagtagctt gaaaaaaggg ggtggactc 1729

The above nucleotide and amino acid sequences are disclosed and further described in  
 U.S. Patent Application Serial No. 09/120,817 to Collmer et al., which is hereby  
 incorporated by reference in its entirety.

A hypersensitive response elicitor protein or polypeptide derived from  
*Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ. ID.  
 No. 11 as follows:

30 Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln  
 1 5 10 15  
 Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser  
 20 25 30  
 Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile  
 35 35 40 45  
 Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly  
 50 55 60  
 Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala  
 65 70 75 80  
 40 Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser  
 85 90 95

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5 Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met  
100 105 110

Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala  
115 120 125

10 Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val  
130 135 140

Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala  
145 150 155 160

Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly  
165 170 175

Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly  
180 185 190

15 Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala  
195 200 205

Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn  
210 215 220

Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp  
225 230 235 240

20 Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn  
245 250 255

Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln  
260 265 270

Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly  
275 280 285

25 Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser  
290 295 300

Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val  
305 310 315 320

30 Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln  
325 330 335

Gln Ser Thr Ser Thr Gln Pro Met  
340

Further information regarding this hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas solanacearum* is set forth in Arlat, M., et al., "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference in its entirety. It is encoded by a DNA molecule from *Pseudomonas solanacearum* having a nucleotide sequence corresponding SEQ. ID. No. 12 as follows:

40 atgtcagtcg gaaacatcca gagcccgctcg aacctcccggt gtctgcagaa cctgaacctc 60  
aacaccaaca ccaacagcca gcaatcggggc cagtcctgtgc aagacctgat caagcaggtc 120  
gagaaggaca tcctcaacat catcgcagcc ctcgtgcaga aggccgcaca gtcggcgggc 180

5 ggcaacaccg gtaacaccgg caacgcgccg gcgaaggacg gcaatgccaa cgcgggcgcc 240  
aacgacccga gcaagaacga cccgagcaag agccaggctc cgcagtcggc caacaagacc 300  
ggcaacgtcg acgacgcca caaccaggat ccgatgcaag cgctgatgca gctgctggaa 360  
gacctggtga agctgctgaa ggcggccctg cacatgcagc agcccggcgg caatgacaag 420  
ggcaacggcg tgggcggtgc caacggcgcc aagggtgccg gcggccaggg cggcctggcc 480  
gaagcgctgc aggagatcga gcagatcctc gcccagctcg gcggcgggcg tgctggcgcc 540  
ggcgggcgcg gtggcggtgt cggcggtgct ggtggcgcg atggcggtc cggtcgggt 600  
ggcgcgaggc gtgcgaacgg cgccgacggc ggcaatggcg tgaacggcaa ccaggcgaac 660  
ggcccgcgaga acgcaggcga tgtcaacggg gccaacggcg cggatgacgg cagcgaagac 720  
10 cagggcgggc tcaccggcgt gctgcaaaag ctgatgaaga tcctgaacgc gctggtgcag 780  
atgatgcagc aaggcggcct cggcgggcg aaccaggcgc agggcggtc gaagggtgcc 840  
ggcaacgcct cgccggcttc cggcgcgaa cggggcgcg accagcccg ttcggcggtat 900  
gatcaatcgt ccggccagaa caatctgcaa tcccagatca tggatgtggt gaaggaggtc 960  
gtccagatcc tgcagcagat gctggcgggc cagaacggcg gcagccagca gtccacctcg 1020  
15 acgcagccga tgtaa 1035

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 5,776,889 to Wei et al., which is hereby incorporated by reference in its entirety.

Other embodiments of the present invention include, but are not limited to, use of hypersensitive response elicitor proteins or polypeptides derived from *Erwinia carotovora* and *Erwinia stewartii*. Isolation of an *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui, et al., "The RsmA Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrpN*<sub>Ecc</sub> and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference in its entirety. A hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference in their entirety.

Hypersensitive response elicitor proteins or polypeptides from various *Phytophthora* species are described in Kaman, et al., "Extracellular Protein Elicitors from *Phytophthora*: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993); Ricci, et

al., "Structure and Activity of Proteins from Pathogenic Fungi *Phytophthora* Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989); Ricci, et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of *Phytophthora parasitica*," Plant Path. 41:298-307 (1992); Baillreul, et al., "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defense Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet, et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference in their entirety.

Another hypersensitive response elicitor protein or polypeptide which can be used in accordance with the present invention is derived from *Clavibacter michiganensis* subsp. *sepedonicus* and is described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference in its entirety.

Fragments of the above hypersensitive response elicitor proteins or polypeptides as well as fragments of full length elicitors from other pathogens can also be used according to the present invention.

Suitable fragments can be produced by several means. Subclones of the gene encoding a known elicitor protein can be produced using conventional molecular genetic manipulation for subcloning gene fragments, such as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), and Ausubel et al. (ed.), Current Protocols in Molecular Biology, John Wiley & Sons (New York, NY) (1999 and preceding editions), which are hereby incorporated by reference in their entirety. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or polypeptide that can be tested for elicitor activity, e.g., using procedures set forth in Wei, Z-M., et al., Science 257: 85-88 (1992), which is hereby incorporated by reference in its entirety.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. Erlich, H.A., et al., "Recent Advances in the Polymerase Chain Reaction," Science 252:1643-51 (1991), which is hereby incorporated by reference in

its entirety. These can then be cloned into an appropriate vector for expression of a truncated protein or polypeptide from bacterial cells as described above.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which elicit a hypersensitive response are fragments of the *Erwinia amylovora* hypersensitive response elicitor protein or polypeptide of SEQ. ID. No. 3. The fragments can be a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, or an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span amino acids 105 and 403 of SEQ. ID. No. 3. The N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 1 and 98, 1 and 104, 1 and 122, 1 and 168, 1 and 218, 1 and 266, 1 and 342, 1 and 321, and 1 and 372. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 76 and 209, 105 and 209, 99 and 209, 137 and 204, 137 and 200, 109 and 204, 109 and 200, 137 and 180, and 105 and 180. DNA molecules encoding these fragments can also be utilized in the chimeric gene of the present invention.

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the

protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The hypersensitive response elicitor proteins or polypeptides used in accordance with the present invention are preferably produced in purified form (preferably at least about 80%, more preferably 90%, pure) by conventional techniques. Typically, the protein or polypeptide of the present invention is secreted into the growth medium of recombinant host cells (discussed *infra*). Alternatively, the protein or polypeptide of the present invention is produced but not secreted into growth medium. In such cases, to isolate the protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to sequential ammonium sulfate precipitation. The fraction containing the hypersensitive response elicitor protein or polypeptide of interest is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the proteins. If necessary, the protein fraction may be further purified by HPLC.

Other hypersensitive response elicitors can be readily identified by isolating putative protein or polypeptide candidates and testing them for elicitor activity as described, for example, in Wei, Z-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference in its entirety. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e., local necrosis) by using them to infiltrate appropriate plant tissues. Once identified, DNA molecules encoding a hypersensitive response elicitor can be isolated using standard techniques known to those skilled in the art.

DNA molecules encoding other hypersensitive response elicitor proteins or polypeptides can also be identified by determining whether such DNA molecules hybridizes under stringent conditions to a DNA molecule having the nucleotide sequence of SEQ. ID. Nos. 2, 4, 6, 8, 10, or 12. An example of suitable stringency conditions is when hybridization is carried out at a temperature of about 37°C using a hybridization medium that includes 0.9M sodium citrate ("SSC") buffer, followed by washing with 0.2x SSC buffer at 37°C. Higher stringency can readily be attained by increasing the temperature for either hybridization or washing conditions

or increasing the sodium concentration of the hybridization or wash medium. Nonspecific binding may also be controlled using any one of a number of known techniques such as, for example, blocking the membrane with protein-containing solutions, addition of heterologous RNA, DNA, and SDS to the hybridization buffer, and treatment with RNase. Wash conditions are typically performed at or below stringency. Exemplary high stringency conditions include carrying out hybridization at a temperature of about 42°C to about 65°C for up to about 20 hours in a hybridization medium containing 1M NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.1% sodium dodecyl sulfate (SDS), 0.2% ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, and 50 µg/ml *E. coli* DNA, followed by washing carried out at between about 42°C to about 65°C in a 0.2x SSC buffer.

The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference in its entirety, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including prokaryotic organisms and eukaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccinia virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which



is hereby incorporated by reference in its entirety), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference in its entirety), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference in its entirety.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promoter which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eukaryotic promoters differ from those of prokaryotic promoters. Furthermore, eukaryotic promoters and accompanying genetic signals may not be recognized in or may not function in a prokaryotic system, and, further, prokaryotic promoters are not recognized and do not function in eukaryotic cells.

Similarly, translation of mRNA in prokaryotes depends upon the presence of the proper prokaryotic signals which differ from those of eukaryotes. Efficient translation of mRNA in prokaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG,

which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference in its entirety.

Promoters vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the P<sub>R</sub> and P<sub>L</sub> promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5 (tac)* promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in prokaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not

limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

5                   Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast,  
10                   mammalian cells, insect, plant, and the like.

                  Because it is desirable for recombinant host cells to secrete the hypersensitive response elicitor protein or polypeptide, it is preferable that the host cell also be transformed with a type III secretion system in accordance with Ham et al., "A Cloned *Erwinia chrysanthemi* Hrp (Type III Protein Secretion) System  
15                   Functions in *Escherichia coli* to Deliver *Pseudomonas syringae* Avr Signals to Plant Cells and Secrete Avr Proteins in Culture," Microbiol. 95:10206-10211 (1998), which is hereby incorporated by reference in its entirety.

                  Isolation of the hypersensitive response elicitor protein or polypeptide from the host cell or growth medium can be carried out as described above.

20                   The methods of the present invention can be performed by treating the fruit or vegetable either prior to or after harvest of the fruit or vegetable.

                  Suitable preharvest application methods include, without limitation, high or low pressure spraying of the entire plant and fruits. Suitable postharvest application methods include, without limitation, low or high pressure spraying,  
25                   coating, or immersion. Other suitable application procedures (both preharvest and postharvest) can be envisioned by those skilled in the art provided they are able to effect contact of the hypersensitive response elicitor polypeptide or protein with the fruit or vegetable. Once treated, the fruits or vegetables can be handled, packed, shipped, and processed using conventional procedures to deliver the produce to  
30                   processing plants or end-consumers.

                  The hypersensitive response elicitor polypeptide or protein can be applied to fruits or vegetables in accordance with the present invention alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor

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polypeptide or protein can be applied separately to fruits or vegetables with other materials being applied at different times.

A composition suitable for treating fruits or vegetables in accordance with the application embodiment of the present invention contains an isolated hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. The composition preferably contains greater than about 500 nM hypersensitive response elicitor polypeptide or protein, although greater or lesser amounts of the hypersensitive response elicitor polypeptide or protein depending on the rate of composition application and efficacy of different hypersensitive response elicitor proteins or polypeptides.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematocide, and mixtures thereof. Suitable fertilizers include  $(\text{NH}_4)_2\text{NO}_3$ . An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and ripening agents. These materials can be used either to facilitate the process of the present invention or to provide additive benefits to inhibit postharvest disease and desiccation.

As indicated above, one embodiment of the present invention involves treating fruits or vegetables with an isolated hypersensitive response elicitor protein or polypeptide. The hypersensitive response elicitor protein or polypeptide can be isolated from its natural source (e.g., *Erwinia amylovora*, *Pseudomonas syringae*, etc.) or from recombinant source transformed with a DNA molecule encoding the protein or polypeptide.

Another aspect of the present invention relates to a DNA construct as well as host cells, expression systems, and transgenic plants which contain the heterologous DNA construct.

The DNA construct includes a DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a plant-expressible promoter operably coupled 5' to the DNA molecule and which is effective to transcribe the DNA molecule in fruit or vegetable tissue, and a 3' regulatory region operably coupled to the DNA molecule. Expression of the DNA molecule in fruit or vegetable

tissue imparts to a fruit or vegetable resistance against postharvest disease or desiccation.

Expression of such heterologous DNA molecules requires a suitable promoter which is operable in plant tissues. In some embodiments of the present invention, it may be desirable for the heterologous DNA molecule to be expressed in many, if not all, tissues. Such promoters yield constitutive expression of coding sequences under their regulatory control. Exemplary constitutive promoters include, without limitation, the nopaline synthase promoter (Fraley et al., Proc. Natl. Acad. Sci. USA 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety) and the cauliflower mosaic virus 35S promoter (O'Dell et al., "Identification of DNA Sequences Required for Activity of the Cauliflower Mosaic Virus 35S Promoter," Nature, 313(6005):810-812 (1985), which is hereby incorporated by reference in its entirety).

While constitutive expression is generally suitable for expression of the DNA molecule, it should be apparent to those of skill in the art that temporally or tissue regulated expression may also be desirable, in which case any regulated promoter can be selected to achieve the desired expression. Typically, the temporally or tissue regulated promoters will be used in connection with the DNA molecule that are expressed at only certain stages of development or only in certain tissues.

In another embodiment of the present invention, expression of the heterologous DNA molecule is directed in a tissue-specific manner or environmentally-regulated manner (i.e., inducible promoters). Tissue-specific promoters under developmental control include promoters that initiate transcription only in certain tissues.

For example, the E4 and E8 promoters of tomato have been used to direct fruit-specific expression of a heterologous DNA sequence in transgenic tomato plants (Cordes et al., Plant Cell 1:1025-1034 (1989); Deikman et al., EMBO J. 7:3315-3320 (1988); and Della Penna et al., Proc. Natl. Acad. Sci. USA 83:6420-6424 (1986), which are hereby incorporated by reference in their entirety). Another fruit-specific promoter is the PG promoter (Bird et al., Plant Molec. Biol. 11:651-662 (1988), which is hereby incorporated by reference in its entirety). Another tissue-specific promoter is the AP2 promoter from the ovule-specific BEL1 gene promoter

described in Reiser et al., Cell 83:735-742 (1995), which is hereby incorporated by reference in its entirety.

Promoters useful for expression in seed tissues include, without limitation, the promoters from genes encoding seed storage proteins, such as napin, cruciferin, phaseolin, and the like (see U.S. Patent No. 5,420,034 to Kridl et al., which is hereby incorporated by reference in its entirety). Other suitable promoters include those from genes encoding embryonic storage proteins.

Promoters useful for expression in leaf tissue include the Rubisco small subunit promoter.

Promoters useful for expression in tubers, particularly potato tubers, include the patatin promoter.

Examples of environmental conditions that may affect transcription by inducible promoters include anaerobic conditions, elevated temperature, or the presence of light. In some plants, it may also be desirable to use promoters which are responsive to pathogen infiltration or stress. For example, it may be desirable to limit expression of the protein or polypeptide in response to infection by a particular pathogen of the plant. One example of a pathogen-inducible promoter is the *gstI* promoter from potato, which is described in U.S. Patent Nos. 5,750,874 and 5,723,760 to Strittmayer et al., which are hereby incorporated by reference in their entirety.

Expression of the DNA molecule in isolated plant cells or tissue or whole plants also utilizes appropriate transcription termination and polyadenylation of mRNA. Any 3' regulatory region suitable for use in plant cells or tissue can be operably linked to the first and second DNA molecules. A number of 3' regulatory regions are known to be operable in plants. Exemplary 3' regulatory regions include, without limitation, the nopaline synthase 3' regulatory region (Fraley, et al., "Expression of Bacterial Genes in Plant Cells," Proc. Nat'l. Acad. Sci. USA, 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety) and the cauliflower mosaic virus 3' regulatory region (Odell, et al., "Identification of DNA Sequences Required for Activity of the Cauliflower Mosaic Virus 35S Promoter," Nature, 313(6005):810-812 (1985), which is hereby incorporated by reference in its entirety).

The promoter and a 3' regulatory region can readily be ligated to the DNA molecule using well known molecular cloning techniques described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Press, NY (1989), which is hereby incorporated by reference in its entirety.

One approach to transforming plant cells with a DNA molecule of the present invention is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford, et al., which are hereby incorporated by reference in their entirety. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells. Other variations of particle bombardment, now known or hereafter developed, can also be used.

Another method of introducing the DNA molecule into plant cells is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies that contain the DNA molecule. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference in its entirety.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm, et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference in its entirety. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the DNA molecule.

Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* previously transformed with the DNA molecule. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

*Agrobacterium* is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences such as a DNA molecule a hypersensitive response elicitor protein or polypeptide can be introduced into appropriate plant cells by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by *Agrobacterium* and is stably integrated into the plant genome. Schell, J., Science, 237:1176-83 (1987), which is hereby incorporated by reference in its entirety.

Plant tissue suitable for transformation include leaf tissue, root tissue, meristems, zygotic and somatic embryos, and anthers.

After transformation, the transformed plant cells can be selected and regenerated.

Preferably, transformed cells are first identified using, e.g., a selection marker simultaneously introduced into the host cells along with the DNA molecule of the present invention. Suitable selection markers include, without limitation, markers coding for antibiotic resistance, such as kanamycin resistance (Fraley, et al., Proc. Natl. Acad. Sci. USA, 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety). A number of antibiotic-resistance markers are known in the art and other are continually being identified. Any known antibiotic-resistance marker can be used to transform and select transformed host cells in accordance with the present invention. Cells or tissues are grown on a selection media containing an



antibiotic, whereby generally only those transformants expressing the antibiotic resistance marker continue to grow.

Once a recombinant plant cell or tissue has been obtained, it is possible to regenerate a full-grown plant therefrom. Thus, another aspect of the present invention relates to a transgenic plant that includes a heterologous DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, wherein the heterologous DNA molecule is under control of a promoter that induces transcription of the DNA molecule fruit or vegetable tissues. Preferably, the DNA molecule is stably inserted into the genome of the transgenic plant of the present invention.

Plant regeneration from cultured protoplasts is described in Evans, et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference in their entirety.

It is known that practically all plants can be regenerated from cultured cells or tissues, including both monocots and dicots.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the DNA molecule encoding the hypersensitive response elicitor protein or polypeptide is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing or by preparing cultivars. With respect to sexual crossing, any of a number of standard breeding techniques can be used depending upon the species to be crossed. Cultivars can be propagated in accord with common agricultural procedures known to those in the field.

With regard to the use of the hypersensitive response elicitor protein or polypeptide in imparting postharvest disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease can be reduced and symptom development can be delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of controlling postharvest disease has the potential for controlling previously untreatable diseases and avoiding the use of infectious agents or environmentally harmful materials.

With respect to desiccation, complete protection against desiccation may not be conferred, but the severity of desiccation can be reduced. Desiccation protection inevitably will depend, at least to some extent, on other conditions such as storage temperatures, light exposure, etc. However, this method of controlling desiccation has the potential for eliminating some other treatments (i.e., use of coating waxes) which may contribute to reduced costs or, at least, substantially no increase in costs.

The methods of the present invention can be used to control a number of postharvest diseases caused by a variety of pathogens. These postharvest diseases and the causative agents which can be treated according to the present invention include, without limitation, the following: *Penicillium* (e.g., *Penicillium digitatum*), *Botrytis* (e.g., *Botrytis cinerea*), *Phytophthora* (e.g., *Phytophthora citrophthora*), and *Erwinia* (e.g. *Erwinia carotovora*).

A further aspect of the present invention relates to a method of enhancing the longevity of fruit or vegetable ripeness.

According to one embodiment, this aspect of the present invention is carried out by treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to enhance the longevity of fruit or vegetable ripeness. Preferably, as noted above, the hypersensitive response elicitor protein or polypeptide is in isolated form. Treating of the fruit or vegetable can be performed either prior to harvest after harvest of the fruit or vegetable, using the techniques described above.

According to another embodiment, this aspect of the present invention is carried out by providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and then growing the transgenic plant or transgenic plant produced from the transgenic plant

seed under conditions effective to enhance the longevity of fruit or vegetable ripeness in a fruit or vegetable harvested from the transgenic plant. This aspect of the present invention may further include applying the hypersensitive response elicitor polypeptide or protein to the fruit or vegetable to enhance the longevity of fruit or vegetable ripeness. Treating of the fruit or vegetable can be performed either prior to harvest or after harvest of the fruit or vegetable, using the techniques described above.

The methods of the present invention can be utilized to treat a wide variety of fruits and vegetables to control postharvest disease or desiccation as well as enhance the longevity of fruit or vegetable ripeness. Fruits and vegetables which can be treated include any edible plant product, particularly those from traditional crop plants, such as seed, root, tuber, stem, leaf, flower, and fruit. Exemplary transgenic fruit plants and fruits that can be treated include, without limitation, apple, pear, peach, nectarine, apricot, plum, cherry, olive, melon, citrus, grape, strawberry, raspberry, blueberry, currant, pineapple, papaya, guava, banana, and kiwi. Exemplary transgenic vegetable plants and vegetables that can be treated include, without limitation, asparagus, potato, sweet potato, bean, pea, chicory, lettuce, parsley, basil, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, spinach, onion, garlic, eggplant, pepper, celery, leek, radish, carrot, squash, pumpkin, zucchini, cucumber, soybean, tobacco, tomato, sorghum, rhubarb, and sugarcane. Exemplary transgenic grain plants and grain products which can be treated include, without limitation, alfalfa, rice, wheat, barley, corn, and rye.

## EXAMPLES

The following examples are provided to illustrate embodiments of the present invention but are by no means intended to limit its scope.

As used in the following Examples, Messenger<sup>®</sup> refers to a product available from Eden Bioscience Corporation (Bothell, Washington), which contains 3% by weight of harpin<sub>Ea</sub> as the active ingredient and 97% by weight inert ingredients. Harpin<sub>Ea</sub> is one type of hypersensitive response elicitor protein from *Erwinia amylovora*, identified herein by SEQ. ID. No. 3.

**Example 1 – Effect of Treating Orange Fruits with Messenger® on Postharvest Orange Storage**

On day 0, Fall-GLO orange fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO<sub>4</sub>, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger® or buffer solutions on oranges were then dried by air, and the treated oranges were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated oranges was then put into a 18°C growth chamber for storage. On day 7, orange fruits were inoculated with *Penicillium digitatum* and *Botrytis cinerea* by spraying a 10<sup>5</sup> cfu/ml suspension on the surface of orange fruit. The above procedure was performed on 40 orange fruits per treatment.

Measurements of disease were conducted on days 20, 24, and 26 following treatment with Messenger® or buffer solution. Grades 0-5 indicate different disease scales -- Grade 0: No symptoms; Grade 1: 1/5 an individual fruit has disease symptoms; Grade 2: 2/5 an individual fruit has disease symptoms; Grade 3: 3/5 an individual fruit has disease symptoms; Grade 4: 4/5 an individual fruit has disease symptoms; Grade 5: whole fruit has disease symptoms. The results of these treatments are set forth in Table 1 below.

Table 1: Reduction of Disease Index in Oranges

Sample	Days After Treatment	Grade						Index	Efficacy	T-test	
		0	1	2	3	4	5			p<0.05	p<0.01
Messenger®	20	33	3	1	0	2	1	0.09	58.14%	yes	yes
Buffer	20	23	8	0	2	6	1	0.22	n/a	-	-
Messenger®	24	25	2	6	4	1	2	0.20	45.21%	yes	yes
Buffer	24	16	7	3	3	4	7	0.37	n/a	-	-
Messenger®	26	19	4	6	4	5	2	0.29	36.96%	yes	yes
Buffer	26	16	3	3	0	7	11	0.46	n/a	-	-

The data listed in Table 1 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Botrytis cinerea* and providing longer storage life. Messenger® treatment can reduce orange disease about 58.14% at 21 days, about 45.21% at 25 days, and 36.97% at 27 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95%

and 99% confidence levels for the results obtained from Messenger treatment<sup>®</sup> and buffer treatment.

**Example 2 – Effect of Treating Tomato (Hot House) Fruits with Messenger<sup>®</sup> on Postharvest Tomato Storage**

On day 0, Hot House tomato fruits were treated by spraying Messenger<sup>®</sup> solution (ca. 15 ug/ml) or buffer solution (5mM KPO<sub>4</sub>, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger<sup>®</sup> or buffer solutions on tomatoes were then dried by air, and the treated tomatoes were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated tomatoes was then put into 18°C growth chamber for storage. On day 7, tomatoes were inoculated with *Penicillium digitatum* and *Botrytis cinerea* by spraying a 10<sup>5</sup> cfu/ml suspension on the surface of tomato fruit. The above procedure was performed on 15 tomatoes fruits per treatment.

Measurements of disease were conducted on days 21 and 27 following treatment with Messenger<sup>®</sup> or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 2 below.

Table 2: Reduction of Disease Index in Tomatoes

Sample	Days After Treatment	Grade						Index	Efficacy	T-test	
		0	1	2	3	4	5			p<0.05	p<0.01
Messenger <sup>®</sup>	21	7	2	2	3	1	0	0.25	58.70%	yes	yes
Buffer	21	3	1	2	1	2	6	0.61	n/a	-	-
Messenger <sup>®</sup>	27	2	2	4	3	2	2	0.49	30.19%	yes	yes
Buffer	27	1	1	2	2	3	6	0.71	n/a	-	-

The data listed in Table 2 above shows that the Messenger<sup>®</sup> was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Botrytis cinerea* and providing longer storage life. Messenger<sup>®</sup> treatment can reduce tomato disease about 58.70% at 21 days and about 30.19% at 27 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment<sup>®</sup> and buffer treatment.

**Example 3 – Effect of Treating Grape Fruits with Messenger® on Postharvest Grape Storage**

On day 0, Red G. Grape fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO<sub>4</sub>, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger® or buffer solutions on grapes were then dried by air, and the treated grapes were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated grapes was then put into a 18°C growth chamber for storage. On day 7, grapes were inoculated with *Penicillium digitatum* and *Botrytis cinerea* by spraying a 10<sup>5</sup> cfu/ml suspension on the surface of grape fruit. The above procedure was performed on about 3700g of grape fruits per treatment.

Measurements of disease were conducted on days 14 and 21 following treatment with Messenger® or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 3 below.

Table 3: Reduction of Disease Index in Grapes

Sample	Days After Treatment	Grade						Index	Efficacy	T-test	
		0	1	2	3	4	5			p<0.05	p<0.01
Messenger®	14	225	99	42	39	21	13	0.20	45.65%	yes	yes
Buffer	14	98	130	91	52	38	48	0.38	n/a	-	-
Messenger®	21	66	83	126	98	39	27	0.42	39.35%	yes	yes
Buffer	21	18	36	64	72	119	137	0.69	n/a	-	-

The data listed in Table 3 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Botrytis cinerea* and providing longer storage life. Messenger® treatment can reduce grape disease by about 45.65% at 14 days and about 39.35% at 21 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment® and buffer treatment.

**Example 4 – Effect of Treating Grapefruit Fruits with Messenger® on Postharvest Grapefruit Storage**

On day 0, FL 33935 grapefruit fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO<sub>4</sub>, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger® or buffer solutions on grapefruits were then dried by air, and the treated grapefruits were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated grapefruit fruits was then put into a 18°C growth chamber for storage. On day 7, grapefruit fruits were inoculated with *Phytophthora citrophthora* by spraying a 10<sup>5</sup> cfu/ml suspension on the surface of grapefruit fruit. The above procedure was performed on 6 grapefruit fruits per treatment.

Measurements of disease were conducted on days 87, 97, 103, and 111 following treatment with Messenger® or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 4 below.

Table 4: Reduction of Disease Index in Grapefruits

Sample	Days After Treatment	Grade						Index	Efficacy	T-test	
		0	1	2	3	4	5			p<0.05	p<0.01
Messenger®	87	5	1	0	0	0	0	0.03	75.00%	yes	yes
Buffer	87	4	1	0	1	0	0	0.13	n/a	-	-
Messenger®	97	5	0	0	1	0	0	0.10	50.00%	yes	yes
Buffer	97	4	0	1	0	1	0	0.20	n/a	-	-
Messenger®	103	4	1	0	0	1	0	0.17	28.57%	yes	yes
Buffer	103	3	2	0	0	0	1	0.23	n/a	-	-
Messenger®	111	4	1	0	0	0	1	0.20	33.33%	yes	yes
Buffer	111	3	1	0	1	0	1	0.30	n/a	-	-

The data listed in Table 4 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Phytophthora citrophthora* and providing longer storage life. Messenger® treatment can reduce grapefruit disease by about 75.00% at 87 days, about 50.00% at 97 days, about 28.57% at 103 days, and about 33.33% at 111 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant

differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment<sup>®</sup> and buffer treatment.

**Example 5 - Effect of Treating Apple (Fuji) Fruits with Messenger<sup>®</sup> on Postharvest Apple Storage**

On day 0, Fuji apple fruits were treated by spraying Messenger<sup>®</sup> solution (ca. 15 ug/ml) or buffer solution (5mM KPO<sub>4</sub>, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger<sup>®</sup> or buffer solutions on apples were then dried by air, and the treated apples were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated apples was then put into a 18°C growth chamber for storage. On day 7, apples were inoculated with *Penicillium digitatum* and *Phytophthora citrophora* by spraying a 10<sup>5</sup> cfu/ml suspension on the surface of apples. The above procedure was performed on 20 apples per treatment.

Measurements of disease were conducted on days 50, 61, 70, 78, and 85 following treatment with Messenger<sup>®</sup> or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 5 below.

Table 5: Reduction of Disease Index in Apples

Sample	Days After Treatment	Grade							Index	Efficacy	T-test	
		0	1	2	3	4	5	p<0.05			p<0.01	
Messenger®	50	20	0	0	0	0	0	0.00	100.00%	yes	yes	
Buffer	50	18	1	1	0	0	0	0.03	n/a	-	-	
Messenger®	61	19	1	0	0	0	0	0.01	88.89%	yes	yes	
Buffer	61	16	2	1	0	0	1	0.09	n/a	-	-	
Messenger®	70	18	0	2	0	0	0	0.04	71.43%	yes	yes	
Buffer	70	14	2	2	1	0	1	0.14	n/a	-	-	
Messenger®	78	15	2	3	0	0	0	0.08	57.89%	yes	yes	
Buffer	78	13	2	2	1	0	2	0.19	n/a	-	-	
Messenger®	85	13	3	1	1	2	0	0.16	40.74%	yes	yes	
Buffer	85	10	5	0	0	3	2	0.27	n/a	-	-	

The data listed in Table 5 above shows that the Messenger<sup>®</sup> was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Phytophthora citrophora* and providing longer storage life.



Messenger<sup>®</sup> treatment can reduce apple disease by about 100.00% at 51 days, 88.89% at 61 days, 71.43% at 70 days, 57.89% at 78 days, and 40.74% at 85 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment<sup>®</sup> and buffer treatment.

**Example 6 - Effect of Treating Apple (Granny Smith) Fruits with Messenger<sup>®</sup> on Postharvest Apple Storage**

On day 0, Granny Smith apple fruits were treated by spraying Messenger<sup>®</sup> solution (ca. 15 ug/ml) or buffer solution (5mM KPO<sub>4</sub>, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger<sup>®</sup> or buffer solutions on apples were then dried by air, and the treated apples were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated apples was then put into a 18°C growth chamber for storage. On day 7, apples were inoculated with *Penicillium digitatum* and *Phytophthora citrophora* by spraying a 10<sup>5</sup> cfu/ml suspension on the surface of apples. The above procedure was performed on 20 apples per treatment.

Measurements of disease were conducted on days 50, 61, 70, 78, and 85 following treatment with Messenger<sup>®</sup> or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 6 below.

Table 6: Reduction of Disease Index in Apples

Sample	Days After Treatment	Grade						Index	Efficacy	T-test	
		0	1	2	3	4	5			p<0.05	p<0.01
Messenger <sup>®</sup>	50	20	0	0	0	0	0	00.00	100.00%	yes	yes
Buffer	50	19	1	0	0	0	0	0.01	n/a	-	-
Messenger <sup>®</sup>	61	13	5	2	0	0	0	0.09	50.00%	yes	yes
Buffer	61	7	9	3	1	0	0	0.18	n/a	-	-
Messenger <sup>®</sup>	70	7	10	3	0	0	0	0.16	36.00%	yes	yes
Buffer	70	2	12	5	1	0	0	0.25	n/a	-	-
Messenger <sup>®</sup>	78	6	10	3	1	0	0	0.19	32.14%	yes	yes
Buffer	78	2	11	5	1	1	0	0.28	n/a	-	-
Messenger <sup>®</sup>	85	7	9	2	1	1	0	0.20	23.08	yes	yes
Buffer	85	4	10	4	1	0	1		n/a	-	-

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The data listed in Table 6 above shows that the Messenger<sup>®</sup> was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Phytophthora citrophora* and providing longer storage life. Messenger<sup>®</sup> treatment can reduce apple disease by about 100.00% at 51 days, 50.00% at 61 days, 36.00% at 70 days, 32.14% at 78 days, and 23.08% at 85 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment<sup>®</sup> and buffer treatment.

#### **Example 7 - Effect of Treating Tomato Fruits with Messenger<sup>®</sup> on Postharvest Tomato Storage**

On day 0, tomato fruits were treated by spraying Messenger<sup>®</sup> solution (ca. 15 ug/ml) or buffer solution (5mM KPO<sub>4</sub>, pH 6.8) on the surface of fruits in a 22°C greenhouse. After the Messenger<sup>®</sup> or buffer solutions on tomatoes were dried by air, the treated tomatoes were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated tomatoes was then put into a 18°C growth chamber for storage. On day 7, tomatoes were inoculated with *Penicillium digitatum* and *Botrytis cinerea* by spraying a 10<sup>5</sup> cfu/ml suspension on the surface of tomatoes. The above procedure was performed on 44 tomatoes per treatment.

Measurements of disease were conducted on days 18, 27, 35, and 42 following treatment with Messenger<sup>®</sup> or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 7 below.

Table 7: Reduction of Disease Index in Tomatoes

Sample	Days After Treatment	Grade							Index	Efficacy	T-test	
		0	1	2	3	4	5	p<0.05			p<0.01	
Messenger®	18	21	18	5	0	0	0	0.13	37.78%	yes	yes	
Buffer	18	11	21	12	0	0	0	0.20	n/a	-	-	
Messenger®	27	16	18	9	1	0	0	0.18	25.00%	yes	yes	
Buffer	27	8	24	8	4	0	0	0.24	n/a	-	-	
Messenger®	35	7	14	13	10	0	0	0.32	16.67%	yes	yes	
Buffer	35	1	16	15	10	2	0	0.38	n/a	-	-	
Messenger®	42	1	10	9	12	9	3	0.52	12.88%	yes	yes	
Buffer	42	0	3	15	10	11	5	0.60	n/a	-	-	

The data listed in Table 7 above shows that the Messenger<sup>®</sup> was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Botrytis cinerea* and providing longer storage life. Messenger<sup>®</sup> treatment can reduce tomato disease by about 37.78% at 18 days, 25.00% at 27 days, 16.67% at 35 days, and 12.88% at 42 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger<sup>®</sup> treatment and buffer treatment.

**Example 8 - Effect of Preharvest and Postharvest Messenger<sup>®</sup> Treatments on Tomato (Sanibel) Fruit Postharvest Storage**

Plots of red and green Sanibel variety tomatoes were grown under either standard conditions or full Messenger<sup>®</sup> treatment over the course of the growing season. The standard conditions, also known as grower's standard, included fungicide treatment sprayed every seven days after transplanting using primarily fungicides containing copper-based active ingredients. The Messenger<sup>®</sup> treatment included six sprays at rate of 2.2 oz of the product per acre.

Red and green fruits were harvested from both the Messenger<sup>®</sup> treated and grower standard plots. It was noted that green tomatoes from the grower standard treatment plots were smaller (i.e. less mature) than green tomatoes from the messenger treated plants.

Harvested fruits were treated as follows:

- (1) Fruits from Messenger<sup>®</sup> treated plots were further treated with Messenger<sup>®</sup> after harvest;
- (2) Fruits from standard plots were treated with Messenger<sup>®</sup> after harvest;
- (3) Fruits from Messenger<sup>®</sup> treated plots received no additional treatment following harvest; and
- (4) Fruits from standard plots received no additional treatment following harvest.

Postharvest treatment of fruits from groups (1) and (2) was carried out by spraying with Messenger<sup>®</sup> at a rate of 20 ppm harpin<sub>Ea</sub> concentration using a

backpack-sprayer at about 30 p.s.i. The fruit were rolled during application to assure full coverage of the spray. The postharvest treated tomatoes were allowed to air dry and then tomatoes from groups (1)-(4) were marked and mixed together in storage in a single layer. Storage temperatures ranged from about 18 to 32°C and light intervals were approximately 12 hours of light and darkness. Tomatoes were checked daily for rot and desiccation for a total of 31 days after harvest. The results are shown in Table 8 below.

Table 8: Affect of Preharvest and Postharvest Treatment on Rot and Desiccation

Group	Ripe- ness	No. Fruit	Days After Harvest							No. Desiccated	%
			14	19	21	22	23	25	31		
(1) Pre/Postharvest Messenger®	Red	5	0	0	0	1	1	1	2	0	60%
	Green	4	0	0	0	0	0	0	0	0	100%
(2) Postharvest Messenger® Only	Red	5	0	0	0	0	0	0	0	4	20%
	Green	4	0	0	0	0	0	0	0	1	75%
(3) Preharvest Messenger® Only	Red	5	0	0	0	0	0	0	2	0	60%
	Green	5	0	0	0	0	0	0	0	0	100%
(4) No Messenger®	Red	5	1	3	1	5	5	5	5	0	0%
	Green	5	0	0	0	0	0	0	0	1	80%

The red tomatoes from group (4) all rotted by day 21. In contrast, all red tomatoes which received some form of Messenger® treatment showed reduced rate of decay and rot. Near the end of the trial a number of tomatoes were observed to have desiccated, exhibiting shriveled skins but no rot. These were included as non-marketable. These results are suggestive that both preharvest and postharvest Messenger® treatments can reduce the level of rotting and desiccation, thereby extending fresh storage time.

#### **Example 9 - Effect of Messenger on Post Harvested Maturity and Fruit Decay on Tomato During Ambient Storage**

The tomatoes were grown under either standard conditions (identified in Example 8) or full Messenger® treatment over the course of the growing season (identified in Example 8) and then hand picked at the time of commercial harvest. Mature green fruit of uniform size (5/6) were collected throughout the field in four replicate samples of 25 fruit per sample, placed directly into fruit bags and transported to a laboratory facility for postharvest treatment and/or analysis. Three different treatment regimen were examined as follows:

- 5
- (1) Fruits from Messenger<sup>®</sup> treated plots received no additional treatment following harvest;
  - (2) Fruits from standard plots were treated with Messenger<sup>®</sup> after harvest;
  - (3) Fruits from standard plots received no additional treatment following harvest.

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Postharvest treatment of fruits from group (2) was carried out by dipping the fruit in a Messenger<sup>®</sup> solution (20 ppm harpin<sub>Ea</sub>). The postharvest treated tomatoes were allowed to air dry and then tomatoes from groups (1)-(3) were marked and mixed together in tomato crates for storage. Storage temperatures ranged from about 23 to 26°C (75-80°F). The tomatoes were then rated for color development and decay over time using the rating scale below.

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<u>Grade</u>	<u>Description</u>
1	Mature Green: When fruit cut in half, no seeds cut; fruit entirely green with no color break;
2	Pink: Initial sign of color break noticed on some areas of fruit; these areas are usually pink;
3	Pink/Red: Intermediate ripening: Fruit is not total red; some pink still remains;
4	Red: Fruit totally red in color;
5	Decay: Some areas of the fruit beginning to break down from decay.

The results of this test are summarized in Table 9 below.

Table 9: Affect of Preharvest and Postharvest Treatment on Maturity and Decay

Group	Days After Treatment	Grade					Index	Efficacy	T-test	
		1	2	3	4	5			p<0.05	p<0.01
1	10	11	6	8	75	0	0.69	7.28%	yes	yes
2	10	5	7	11	77	0	0.72	3.81%	yes	yes
3	10	5	3	6	86	1	0.75	N/A	N/A	N/A
1	14	4	5	5	86	0	0.75	2.61%	yes	yes
2	14	2	6	5	87	0	0.75	1.57%	yes	yes
3	14	2	4	4	89	1	0.77	N/A	N/A	N/A
1	17	0	0	3	92	5	0.80	3.37%	yes	yes
2	17	0	1	4	82	13	0.81	2.16%	yes	yes
3	17	0	0	1	82	17	0.83	N/A	N/A	N/A
1	20	0	0	0	89	11	0.82	2.61%	yes	yes
2	20	0	0	0	80	20	0.84	0.47%	yes	yes
3	20	0	0	1	76	23	0.84	N/A	N/A	N/A

The data generated in this trial indicate that treatment of tomatoes with Messenger®, either through field sprays or as a post harvest dip, resulted in earlier fruit red ripening compared to grower's standard. In addition, although early ripening was observed, the Messenger® treatments maintained the red ripe condition longer than the grower's standard with delay of breakdown and decay.

**Example 10 - Effect on Messenger on Post Harvested Maturity and Fruit Decay of Tomato Under Cold Storage Conditions**

The tomatoes were grown under either standard conditions (identified in Example 8) or full Messenger® treatment over the course of the growing season (identified in Example 8) and then hand picked at the time of commercial harvest. Mature green fruit of uniform size (5/6) were collected throughout the field in four replicate samples of 25 fruit per sample, placed directly into fruit bags and transported to a laboratory facility for postharvest treatment and/or analysis. Four different treatment regimen were examined as follows:

- (1) Fruits from Messenger® treated plots received no additional treatment following harvest;
- (2) Fruits from Messenger® treated plots were further treated with Messenger® after harvest;

- (3) Fruits from standard plots were treated with Messenger<sup>®</sup> after harvest; and
- (4) Fruits from standard plots received no additional treatment following harvest.

5 Postharvest treatment of fruits from groups (2) and (3) were carried out by dipping the fruit in a Messenger<sup>®</sup> solution (20 ppm harpin<sub>Ea</sub>). The postharvest treated tomatoes were allowed to air dry and then tomatoes from groups (1)-(4) were marked and mixed together in tomato crates for storage in a Custom Packing House cooler at 11°C (52°F). The tomatoes were then rated for color development and decay over time using the rating scale described in Example 8. The results of this study appear in Table 10 below.

Table 10: Affect of Preharvest and Postharvest Treatment on Maturity and Decay

Group	Days After Treatment	Grade					Index	Efficacy	T-test	
		1	2	3	4	5			p<0.05	p<0.01
1	7	66	34	0	0	0	0.27	0.00%	yes	yes
2	7	67	33	0	0	0	0.27	0.75%	yes	yes
3	7	76	24	0	0	0	0.27	7.46%	yes	yes
4	7	68	30	2	0	0	0.27	N/A	yes	yes
1	10	59	31	8	0	0	0.30	7.53%	yes	yes
2	10	60	28	12	0	0	0.30	5.00%	yes	yes
3	10	65	35	0	0	0	0.27	15.63%	yes	yes
4	10	49	42	9	0	0	0.32	N/A	N/A	N/A
1	17	19	35	28	18	0	0.49	7.20%	yes	yes
2	17	20	38	28	14	0	0.47	10.61%	yes	yes
3	17	19	28	39	14	0	0.50	6.06%	yes	yes
4	17	17	27	31	25	0	0.53	N/A	N/A	N/A
1	21	11	28	29	32	0	0.56	6.62%	N/A	N/A
2	21	15	26	37	22	0	0.53	11.92%	yes	yes
3	21	10	33	35	22	0	0.54	10.93%	yes	yes
4	21	10	18	32	40	0	0.60	N/A	N/A	N/A
1	26	3	15	23	59	0	0.68	-2.26%	yes	yes
2	26	9	19	25	41	6	0.63	4.39%	yes	yes
3	26	3	23	31	43	0	0.63	5.00%	yes	yes
4	26	2	19	23	50	1	0.66	N/A	N/A	N/A
1	32	3	15	23	59	0	0.68	-2.26%	yes	yes
2	32	9	19	25	41	6	0.63	4.39%	yes	yes
3	32	3	23	31	43	0	0.63	5.00%	yes	yes
4	32	2	19	23	50	1	0.66	N/A	N/A	N/A

Table 10 cont.

Group	Days After Treatment	Grade					Index	Efficacy	T-test	
		1	2	3	4	5			p<0.05	p<0.01
1	38	0	4	10	84	2	0.77	0.26%	yes	yes
2	38	1	10	15	65	9	0.74	3.64%	yes	yes
3	38	1	5	14	78	2	0.75	2.60%	yes	yes
4	38	0	3	13	80	4	0.77	N/A	N/A	N/A
1	45	0	3	11	74	12	0.79	2.95%	yes	yes
2	45	1	4	12	69	14	0.78	3.93%	yes	yes
3	45	0	1	11	81	7	0.79	3.19%	yes	yes
4	45	0	0	10	73	17	0.81	N/A	N/A	N/A
1	50	0	3	10	63	23	0.82	3.55%	yes	yes
2	50	0	4	11	58	27	0.82	3.55%	yes	yes
3	50	0	0	8	78	14	0.81	4.02%	yes	yes
4	50	0	0	3	71	26	0.85	N/A	N/A	N/A
1	55	0	0	0	73	27	0.85	1.84%	yes	yes
2	55	0	0	0	68	32	0.86	0.69%	yes	yes
3	55	0	0	2	80	18	0.83	4.37%	yes	yes
4	55	0	0	0	65	35	0.87	N/A	N/A	N/A
1	60	0	0	0	65	35	0.87	2.47%	yes	yes
2	60	0	0	0	63	37	0.87	2.02%	yes	yes
3	60	0	0	0	74	26	0.85	4.48%	yes	yes
4	60	0	0	0	54	46	0.89	N/A	N/A	N/A
1	65	0	0	0	53	47	0.89	1.76%	yes	yes
2	65	0	0	0	58	42	0.88	2.86%	yes	yes
3	65	0	0	0	65	35	0.87	4.40%	yes	yes
4	65	0	0	0	45	55	0.91	N/A	N/A	N/A

5 In previous trials when tomatoes were treated with Messenger® in the field and/or with a post harvest dip, the fruit appeared to develop to red ripe more quickly than the grower's standard, when held at ambient temperatures (75-80°F). Although this early ripening was observed, these red fruit did not begin to decay earlier than the grower's standard. In this study, the fruit were held at a constant 52°F in a commercial cold storage room at a tomato packinghouse facility. It appears that 10 this lower temperature slows the ripening process, as would be expected, and Messenger® treatments did not increase the rate of the red ripening for the first 30 days, as observed in previous tests. The Messenger® treatments did, however,



seem to maintain the red ripe condition longer than the grower's standard without breakdown and decay.

**Example 11 - Effect of Messenger on Post Harvested Maturity and Fruit Decay on Tomato**

The tomatoes were grown under either standard conditions (identified in Example 8) or full Messenger<sup>®</sup> treatment over the course of the growing season (identified in Example 8) and then hand picked at the time of commercial harvest. Mature green fruit of uniform size (5/6) were collected throughout the field in four replicate samples of 25 fruit per sample, placed directly into fruit bags and transported to a laboratory facility for postharvest treatment and/or analysis. Four different treatment regimen were examined as follows:

- (1) Fruits from Messenger<sup>®</sup> treated plots received no additional treatment following harvest;
- (2) Fruits from Messenger<sup>®</sup> treated plots were further treated with Messenger<sup>®</sup> after harvest;
- (3) Fruits from standard plots were treated with Messenger<sup>®</sup> after harvest; and
- (4) Fruits from standard plots received no additional treatment following harvest.

Postharvest treatment of fruits from groups (2) and (3) were carried out by dipping the fruit in a Messenger<sup>®</sup> solution (20 ppm harpin<sub>EA</sub>). The postharvest treated tomatoes were allowed to air dry and then tomatoes from groups (1)-(4) were marked and mixed together in tomato crates for storage. Storage temperatures ranged from about 23 to 26°C (75-80°F). The tomatoes were then rated for color development and decay over time using the commercial rating scale from the Florida Tomato Committee color guide as follows:

**Grade Description**

- |   |  |
|---|--|
| 1 | Green: When fruit cut in half, no seeds cut; fruit entirely green with no color break;                   |
| 2 | Breakers: Initial sign of color break on 10% or less of the area of fruit; these areas are usually pink; |
| 3 | Turning: Pink or red on 10 to 30% of the fruit surface;  |

- 4 Pink: Pink or red on 30 to 60% of the fruit surface;
- 5 Light Red: Pink on over 60% of fruit surface and red color no more than 90% of fruit surface;
- 6 Red: Fruit totally red in color; and
- 5 7 Decay: Some areas of the fruit beginning to break down from decay.

The results of this treatment are set forth in Table 11 below.

Table 11: Affect of Preharvest and Postharvest Treatment on Maturity and Decay Data

Group	Days After Treatment	Grade							Index	Efficacy	T-test	
		1	2	3	4	5	6	7			p<0.05	p<0.01
1	3	80	18	2	0	0	0	0	0.17	0.00%	no	no
2	3	73	17	9	1	0	0	0	0.20	-13.11%	yes	yes
3	3	78	19	3	0	0	0	0	0.18	-2.46%	yes	yes
4	3	80	18	2	0	0	0	0	0.17	N/A	no	no
1	7	36	23	22	12	5	2	0	0.33	3.72%	yes	no
2	7	37	23	17	19	4	0	0	0.33	4.96%	yes	no
3	7	40	17	15	18	9	1	0	0.35	0.00%	yes	no
4	7	35	22	19	15	8	1	0	0.35	N/A	no	no
1	14	2	5	8	8	13	65	0	0.74	8.02%	yes	yes
2	14	2	3	5	9	8	72	1	0.77	4.44%	yes	yes
3	14	4	4	7	8	17	60	0	0.73	9.41%	yes	yes
4	14	0	0	6	5	13	72	4	0.80	N/A	no	no
1	17	0	0	2	3	6	89	0	0.83	2.51%	yes	yes
2	17	1	1	1	0	7	88	2	0.83	2.35%	yes	yes
3	17	1	2	0	0	9	88	0	0.83	3.18%	yes	yes
4	17	0	0	0	0	7	89	4	0.85	N/A	no	no
1	21	0	0	0	0	0	97	3	0.86	1.31%	yes	yes
2	21	0	0	0	0	0	97	3	10.86	1.31%	yes	yes
3	21	0	0	0	0	3	95	2	0.86	1.96%	yes	yes
4	21	0	0	0	0	1	87	12	0.87	N/A	no	no
1	28	0	0	0	0	0	85	15	0.88	2.84%	yes	yes
2	28	0	0	0	0	0	91	9	0.87	3.79%	yes	yes
3	28	0	0	0	0	0	81	19	0.88	2.21%	yes	yes
4	28	0	0	0	0	0	67	33	0.90	N/A	no	no
1	32	0	0	0	0	0	22	78	0.97	2.16%	yes	yes
2	32	0	0	0	0	0	16	84	0.98	1.30%	yes	yes
3	32	0	0	0	0	0	55	45	0.92	6.93%	yes	yes
4	32	0	0	0	0	0	7	93	0.99	N/A	no	no

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Table 11 Cont.

Group	Days After Treatment	Grade							Index	Efficacy	T-test	
		1	2	3	4	5	6	7			p<0.05	p<0.01
1	37	0	0	0	0	0	14	86	0.98	1.15%	yes	yes
2	37	0	0	0	0	0	7	93	0.00	0.14%	yes	yes
3	37	0	0	0	0	0	9	91	0.99	0.43%	yes	yes
4	37	0	0	0	0	0	6	94	0.99	N/A	no	no
1	42	0	0	0	0	0	12	88	0.98	1.01%	yes	yes
2	42	0	0	0	0	0	7	93	0.99	0.29%	yes	yes
3	42	0	0	0	0	0	8	92	0.99	0.43%	yes	yes
4	42	0	0	0	0	0	5	95	0.99	N/A	no	no
1	45	0	0	0	0	0	8	92	0.99	0.57%	no	no
2	45	0	0	0	0	0	4	96	0.99	0.00%	no	no
3	45	0	0	0	0	0	4	96	0.99	0.00%	no	no
4	45	0	0	0	0	0	4	96	0.99	N/A	no	no
1	50	0	0	0	0	0	7	93	0.99	0.43%	no	no
2	50	0	0	0	0	0	4	96	0.99	0.00%	no	no
3	50	0	0	0	0	0	4	96	0.99	0.00%	no	no
4	50	0	0	0	0	0	4	96	0.99	N/A	no	no

In previous trials tomatoes treated with Messenger<sup>®</sup> in the field and/or with a post harvest dip appeared to develop to red ripe more quickly, but decayed slower than the grower's standard. The data generated from this trial support these observations. By twenty-one days post harvest, 97% of the Messenger<sup>®</sup> treated tomatoes were full red ripe, compared to 87% of the grower's standard. Although it may be assumed that fruit which reach maturity more quickly will also start to break down more quickly, the results of the present Examples surprisingly demonstrate that these earlier-maturing tomatoes were actually 15% slower to decay than the grower's standard tomatoes. This phenomenon should be of great interest of several segments of the tomato market. The growers may be able to reduce ethylene gashouse timings, and the retail market should be able to significantly reduce inventory shrinkage.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.